

Levels of Chlorophyll a, Chlorophyll b and total Chlorophyll pigment in Sresponse to Heat Stress in *Eruca sativa* plant

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Abstract

A field experiment was conducted in the greenhouse conditions in the Department of Biology at the College of Education - University of Samarra, In this experiment, we evaluated the effect of a number of stresses and their effect on the levels and activity of chlorophyll.a chlorophyll.b and total chlorophyll, The stresses that were dealt with were temperature. In temperature stress we used variable levels of temperatures 4c°, 30c°, 35c° and 40c° and the result were com as flow, an clear and significant increase in levels of chlorophyll a,b and total chlorophyll, It was 0.4853 µg Chl / mg fw at 4c°, 0.31067 µg Chl / mg fw at 4c° and 0.7963 mg total/g tw at 4c° respectively.

Keyword 1, Environment Stress, temperature level. total chlorophyll, Eruca sativa

1. INTRODUCTION

Heat stress is considered as one of the biggest problems facing humanity with regard to plant production and problems related to global warming. Its also defined as the increase in temperature beyond a threshold level to which a plant is exposed and causes irreparable damage [1]. Many authors like mentioned that higher plants have a moderate thermal growth level, as the minimum temperature for their growth ranges between 10 to 30 degrees Celsius. Three types of heat stresses experienced by plants, 1st temperatures below freezing, 2nd low temperatures above freezing, and 3rd high temperatures [2]. In the plant genome, there is a group of genes related to heat stress, as these genes are activated and activated upon the formation of ROS resulting from heat shock, as these factors are among the inducers of the initiation of the activity of these genes, which ultimately leads to the production of shock proteins[3]. In general, it appears that chlorophyll b is clearly affected by temperature fluctuations, especially when the temperature drops sharply. Thermal adaptations take place inside the plant specifically in chloroplasts., which in turn leads to a decrease in biomass and productivity, the heat stress in plants is evident in two points, the first is the change the activation of chlorophyll and the second is the generation of ROS [4].

2.MATERIALS AND METHODS

2.1 Standard growth conditions of plant

Specific controlled conditions was used to growth the plant (*Eruca sativa* Mill), we can summarized this conditions by the following : (14h light with~54µE, and 21 °C / 10h and 20 °C, 55-60 % relative humidity) on a 2:1:1 petamoss, perlite and vermiculite. Flat containers with high edge with cultured pots were transferred to controlled environmental conditions. After 6 weeks, the plants were directly exposed to one by one to the following abiotic stresses (drought, salinity, light and temperature) with different time points of exposure depend on the method and type of abiotic stress, on other hand specific groups were left or kept at growth chamber conditions without any changes.

2.2 Heat shock.

"Plants were cultivated at 21 °C for the first 42 days, when they were 6-weeks-old. To prepare the plants for heat shock, we began by moving them to develop at certain temperatures: 4°C (cold), , 30°C, and 35°C, 40°C. The collection of leaf samples began after 2 hours, 4 hours, and 6 hours of incubation at the optimal temperature, as indicated by [5].

3.3 Quantification of chlorophylls

To get the most accurate calculation for chlorophyll measurement, 20mg tissue samples were ground in liquid nitrogen and homogenized in 1 ml of 80% acetone. By using vortex for almost (5) mints, samples suspension was mixed and followed by incubation for 1h in darkness.

The samples centrifuged at 13000 rpm for 15 min and the supernatants were collected in new tubes to use for measurement. Chlorophyll content in aqueous acetone was quantified spectrophotometrically by measuring the absorbance of the extract at 646.6 nm and 663.6 nm. The total chlorophyll content (chlorophyll a and b) in each sample was calculated according to [6].

- Total chlorophyll [$\mu\text{g}/\text{mg}$] = $(17.76(A_{646.6}) - 7.34 (A_{663.6})) / \text{fresh weight}$
- Chl a [$\mu\text{g}/\text{mg}$] = $(12.25(A_{663.6}) - 2.55(A_{646.6})) / \text{fresh weight}$
- Chl b [$\mu\text{g}/\text{mg}$] = $(20.31(A_{646.6}) - 4.91 (A_{663.6})) / \text{fresh weight}$

Cold methanol /HCl/ water (90:1:1, vol) was used as buffer to extract Anthocyanins with the same procedure as that for the chlorophyll measurements as described by Sims and [7].

Anthocyanins concentration was estimated as the following:

- Anthocyanins ($\mu\text{mol ml}^{-1}$) = $A_{529} - (0.288 A_{650})$
- Chl a ($\mu\text{mol ml}^{-1}$) = $0.01373 A_{663} - 0.000897 A_{537} - 0.003046 A_{647}$
- Chl b ($\mu\text{mol ml}^{-1}$) = $0.02405 A_{647} - 0.004305 A_{537} - 0.005507 A_{663}$

While the total carotenoides in the tissues, founded using the following equation.

3. RESULTS AND DISCUSSIONS

3.1 Effects of Temperature on chlorophyll a.

The concentration of chlorophyll.a increased at all levels of temperature, and we note that the temperature of 4°C was the most affected, especially after (6 hours) of exposure, as this level of heat continued with significant results at all times of exposure. Then came the temperature of 35°C, where a clear effect was shown and a noticeable increase in the levels of chlorophyll b especially at (6 hours) of exposure . As for the other thermal levels, it was less responsive, but the results were significant.

Table.1 Effect of temperature stress 4°,30°,35° and 40° for different periods of time on chlorophyll. a concentration in *Eruca sativa* mill.

Time/h Temp / c°	2/h	4/h	6/h	Control
4 c°	0.3230 d	0.4350 b	0.4853 a	0.2613 f
30 c°	0.2873 ef	0.3280 de	0.4063 bc	0.2613 f
35 c°	0.3507 cd	0.3907 bc	0.4437 b	0.2613 f
40 c°	0.3230 de	0.2943 ef	0.3410 d	0.2613 f

It seems that the plant responds well to lower temperatures and not the other way around, as we say that the plant moves to the stage of tissue damage and then death at 40°C, These results are consistent with [8]. As we mentioned before, thermal adaptations take place inside the plant specifically in chloroplasts., which in turn leads to a decrease in biomass and productivity, the heat stress in plants is evident in two points, the first is the change the activation of chlorophyll.a and the second is the generation of ROS [9].

3.2 Effects of Temperature on chlorophyll b.

A sharp rise in chlorophyll b concentrations in relation to a temperature of 4°C Celsius, and this was repeated in chlorophyll, as all time periods of exposure showed a clear response to the plant in terms of increased concentrations of chlorophyll b, but the greatest response appeared at 6 hours of treatment, Regarding the two high temperature cyclists, 30°C, 35°C, and 40°C, an increase in concentrations was observed at (6 hours) of treatment and (4 hours), but at (2 hours) of treatment the plant did not show a clear response. In general, it appears that chlorophyll b is clearly affected by temperature fluctuations, especially when the temperature drops sharply. These results are consistent with [10].The plant adopts a series of mechanisms through which it maintains the safety of its members from the danger of heat shock, which includes maintenance of membrane stability, scavenging of ROS, production of antioxidants[11].

Table.2 Effect of temperature stress 4°,30°,35° and 40° for different periods of time on chlorophyll b concentration in *Eruca sativa* mill.

Time/h Temp / c°	2/h	4/h	6/h	Control
4 c°	0.4987 e	0.6603 b	0.7963 a	0.3157 h
30 c°	0.3680 g	0.4507 f	0.5537 cd v	0.3157 h
35 c°	0.3967 g	0.5307 d	0.5700 c	0.3157 h
40 c°	0.3673 g	0.3027 h	0.3070 h	0.3157 h

3.3 Effects of Temperature on total chlorophyll

The results of total chlorophyll appear to be identical with the results of chlorophyll a and b, and that the sharp rise in the concentrations of total chlorophyll at temperature 4°C, especially at the level of 6 hours, and the results are gradual at 4-hours and 2-hours. It seems that the increase in the exposure time has a direct effect on the increase in concentrations, and we find that the 6-hour period has witnessed the highest levels of response. The temperature of 30°C and 35°C has also witnessed a significant effect on the content of chlorophyll, while the 40 degree did not show any significant response at the level of 6-hours, 4-hours. We believe that the plant faces tissue damage and great damage to the metabolic process of the plant in general and at the cellular level in particular.

Table.3 Effect of temperature stress 4°,30°,35° and 40° for different periods of time on total chlorophyll concentration in *Eruca sativa* mill.

Time/h Temp / c°	2/h	4/h	6/h	Control
4 c°	0.4987 e	0.6603 b	0.7963 a	0.3157 h
30 c°	0.3680 g	0.4507 f	0.5537 cd	0.3157 h
35 c°	0.3967 g	0.5307 d	0.5700 c	0.3157 h
40 c°	0.3673 g	0.3027 h	0.3070 h	0.3157 h

This enables the alteration of biochemical composition as well as the induction of microalgal growth and biomass generation to enhance chlorophyll accumulation, it has been proposed to modulate microalgal culture under controlled circumstances[12, 13].

4. CONCLUSION

In conclusion, while temperature stress generally tends to reduce chlorophyll content in plants, the extent and specifics of this response can vary widely depending on the plant species, the severity and duration of stress, and other environmental factors. Understanding these nuances is crucial for predicting and managing the impact of temperature stress on plant productivity and ecosystem dynamics.

5. REFERENCES

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